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FACTORS AFFECTING OXYGEN CONSUMPTION IN THE MARINE PULMONATE *AMPHIBOLA CRENATA* (GMELIN, 1791)

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Abstract

The rate of oxygen consumption by *Amphibola crenata* is unaffected by the salinity of the external medium in the range 0–125% sea water. There is no significant difference between the rates of oxygen consumption in air and water, oxygen consumption varying with the 0.45 power of body weight. During exposure to anaerobic conditions the snails showed an increase in the post-anaerobic:pre-anaerobic respiratory rate, this ratio reaching a maximum after 6 h anoxia over the range 0–125% sea water. Specimens of *Amphibola crenata* exposed to declining oxygen tensions at full salinity show almost no ability to regulate their rate of oxygen consumption. The ratio K_1/K_2, (see text) plotted against the weight specific oxygen consumption yields the following equation: $K_1/K_2 = 527.7\ QO_2^{1.610}$. As salinity decreases, the ratio K_1/K_2 also decreases, indicating an increase of oxygen independence with decreasing salinities. As salinity decreases, the zone of critical pressure decreases, *i.e.* the snails become more oxygen independent; the maximum decrease in the zone of critical pressure coming in animals exposed to 0 and 25% sea water. When exposed to both salinity and anoxic stress, the animals reach their maximum degree of oxygen independence.

Introduction

Amphibola crenata (Gmelin, 1791) is a pulmonate gastropod and a monotypic species of the family Amphibolidae, endemic to New Zealand. The snail belongs to a group of pulmonates sometimes termed "gehydrophilous" in which, while the gill has been replaced by a lung, the animal has not yet become truly an inhabitant of fresh water (Farnie, 1919). Morton and Miller (1968) have described *Amphibola* as an archaic pulmonate, still having an operculum and veliger larva, and place it at a "lung fish" stage of gastropod evolution.

Amphibola is found in vast numbers on mud flats in sheltered bays, inlets, and estuaries. Wherever it occurs, especially on mud flats, the snail is usually the most noticeable and dominant animal. In spite of this, very little work has been done concerning the animal's ecology and none concerning its physiology. Watters (1964) published a detailed study of the distribution of *Amphibola* in the area used in the present study and recorded snails in salinities ranging from 3–30.5‰ with the majority in salinities of 22–25‰. Personal observations indicate that *Amphibola* occurs in the study area in salinities ranging from 0–40‰.

Amphibola is a detritus feeder. Watters (1964) reported that the snails tend

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Abbreviations: VO_2 = rate of oxygen uptake, QO_2 = weight specific oxygen consumption, PO_2 = oxygen tension, K_1/K_2 = ratio indicating oxygen dependence, P_c = zone of critical pressure (for *A. crenata* oxygen cosumption).

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to burrow under mud or sand as the tide rises and remain there until the tide recedes. Briggs (1972) found that *A. crenata* is submerged for only approximately 1–2 h in any one tidal cycle. Thus, the animals may be exposed to anoxic conditions for up to about 2 h. Over a tidal cycle, the oxygen concentration of the ambient water may drop by as much as 75% (personal observation). In addition, in many cases, the snails are not covered by sea water during each tidal cycle and may remain exposed for several weeks.

While many studies deal with effects of salinity on respiration in marine invertebrates and several with effects of declining oxygen tension, only Bayne (1973) has examined the combined effects of salinity and declining oxygen tension on respiration.

Amphibola occupies a transitional habitat between marine and terrestrial conditions and is one of the very few holoeuryhaline species known. Thus, the present study was undertaken to compare its respiratory capabilities with those of marine and freshwater gastropods.

Materials and Methods

Specimens of *Amphibola crenata* (Gmelin, 1971), 0.004–1.0 g dry weight (approximately 0.75–3.5 cm shell height) were collected from Hoopers Inlet, Portobello, New Zealand, and maintained in the laboratory at 15°C for at least 1 week before experiments. Animals were used within three weeks of collection. Shell height and dry tissue weight were determined for 75 freshly collected snails. The linear regression relating shell height to tissue dry weight gave the equation: $Y = 0.022X^{3.01}$ where Y = dry weight of tissue (g), X = shell height (cm), and ($r = 0.961$; $p < 0.001$). After periods of 6 and 12 weeks these same measurements were taken on another 10 animals. There was no evidence of significant weight loss even after 12 weeks in the laboratory.

Aquatic oxygen consumption of *A. crenata* was measured using a Radiometer oxygen electrode according to the method described by Crisp *et al.* (1978). Aerial oxygen consumption was measured using a Gilson differential respirometer. In all cases, individual animals were used for each experiment and experimental chambers ranging from 5–50 ml were used in the aquatic respiration determinations. These chambers were cleaned frequently with hot water and bleach to eliminate bacterial growth. A control (chamber with aerated sea water) was run after every fifth experiment.

For experiments on the effects of salinity on oxygen uptake, Otago Harbour sea water (salinity $\cong$ 33.5‰) was diluted with rainwater or evaporated to give the appropriate salinity. Animals were placed directly from 100% sea water into the experimental salinity. Therefore, the short-term or acute response to salinity change is reported here. Snails were immediately active upon direct transfer to the salinity range 25–125% sea water. However, animals placed directly into rainwater did not emerge from their shells for the first 2 h. Therefore, animals were placed in 0% sea water for 2 h before the start of the experiments. All experiments were carried out at 15°C.

To determine the effects of declining oxygen tension upon oxygen uptake, the snails were placed in the respirometer chamber and allowed to exhaust the available oxygen. While it may be argued that this method can introduce interference by other metabolites in the respiratory response, it was felt that this method reflected conditions likely to be experienced in the natural environment, where the snails are not likely to experience a sudden decline in available oxygen. In addition, Sassaman

and Mangum (1972) simultaneously monitored the oxygen consumption of several species of anemones in closed containers and the pH of the water and found that detectable increases in metabolites were confined to oxygen levels below about 25 mm Hg (approximately 16% air saturation). They concluded that the response of animals exposed to decreasing oxygen tension in sealed containers was due essentially to changes in the oxygen tension. Taylor and Brand (1975a, b) also used the closed chamber technique and found oxygen consumption rates unaffected by metabolite accumulation in bivalves.

For experiments to determine "oxygen debt" or the effect of prolonged exposure to anoxia, animals were allowed to deplete the oxygen supply and then left under anaerobic conditions for different periods, after which the water in the chamber was replaced with well-aerated sea water. Rates of oxygen uptake were again measured or the animals again allowed to deplete the oxygen supply.

For experiments to determine the effects of anoxia and salinity on oxygen uptake in declining oxygen tensions, snails of a narrow size range were used. Variations in the data due to weight differences were minimized by relating all measurements of rate of oxygen uptake (VO_2) to $W^{0.45}$ where W is dry weight (g) and 0.45 is the value of the exponent relating VO_2 to body size ($VO_2 = aW^{0.45}$) (Bayne and Livingston, 1977).

Results

Effect of salinity on oxygen uptake at full oxygen tension

Table I shows the relationship between oxygen consumption in air-saturated sea water and dry body weight, together with the relationship between oxygen consumption in air and dry body weight. The data have been fitted to the equation $Y = aX^b$, where Y = oxygen consumption in ml/h (VO_2) and X = dry weight in g. The equation parameters are given in Table I. While it appears that oxygen uptake rates are higher in water than in air, the difference is not significant.

The effect of salinity on the rate of oxygen uptake (VO_2), and the equation parameters are also given in Table I. There is no significant difference between any of the lines; however, the rate in 125% sea water appears slightly lower than the rate in other salinities. It is possible that with a large sample size these lines could be separated.

Oxygen debt—the effect of hypoxia on oxygen consumption

The effects of anoxia on the rate of oxygen consumption at different experimental salinities are expressed in Table II in terms of a possible oxygen debt, *i.e.*

Table I

Linear regression equation of oxygen consumption (log 10^Y) on dry weight (log$^{X\,g}$); n = number of determinations; r = correlation coefficient. See text for details.

Experimental conditions	a	b	n	*r*	*P*
Aerial	0.138	0.46	42	0.56	0.001
125% SW	0.108	0.43	30	0.84	0.001
100% SW	0.158	0.45	70	0.89	0.001
75% SW	0.139	0.44	30	0.88	0.001
50% SW	0.134	0.45	30	0.60	0.001
25% SW	0.128	0.46	35	0.88	0.001
0% SW	0.141	0.43	25	0.86	0.001

TABLE II

Effect of salinity on the "oxygen debt" in Amphibola crenata *exposed to zero oxygen tension. N = 10 for each determination. Oxygen debt as (VO_2 after hypoxia ÷ VO_2 before hypoxia) ± SD*

% Seawater	Hours hypoxia				
	2	4	6	12	24
125	1.04 ± 0.10	1.48 ± 0.02	1.51 ± 0.10	1.61 ± 0.09	1.69 ± 0.13
100	0.93 ± 0.21	1.48 ± 0.06	1.59 ± 0.04	1.64 ± 0.10	1.76 ± 0.05
75	1.01 ± 0.06	1.31 ± 0.11	1.40 ± 0.04	1.61 ± 0.06	1.80 ± 0.10
50	1.24 ± 0.10	1.51 ± 0.15	1.41 ± 0.08	1.81 ± 0.11	1.69 ± 0.06
25	1.61 ± 0.07	1.70 ± 0.11	1.68 ± 0.08	1.76 ± 0.12	1.79 ± 0.08
0	1.58 ± 0.30	1.53 ± 0.07	1.69 ± 0.10	1.76 ± 0.08	1.74 ± 0.05

the ratio of post-anoxia VO_2 to pre-anoxia VO_2. Values notably greater than one indicate a higher level of oxygen consumption when animals were returned to oxygen-saturated sea water than before exposure to anoxic conditions. Effects of salinity were most pronounced in animals exposed to 2 h hypoxia. Animals in 125%, 100% (control), and 75% sea water showed no major oxygen debt after 2 h hypoxia, whereas animals in more dilute salinities (50, 25, and 0% sea water) showed large oxygen debts. Maximum values were attained in all salinities after 12–24 h exposure to hypoxic conditions.

Oxygen dependence and independence

Several studies concern the effects of declining oxygen tension on oxygen uptake in marine invertebrates and the treatment of data derived from these experiments (Tang, 1933; Bayne, 1971a; Mangum and Van Winkle, 1973; Bayne and Livingston, 1977).

Tang (1933) and Bayne (1971a) used the following simple hyperbolic expression to describe the relationship between the rate of oxygen uptake and ambient oxygen tension:

$$QO_2 = PO_2 \div (K_1 + (K_2 \cdot PO_2)) \quad (1)$$

where: QO_2 = weight specific oxygen consumption (ml/(h/g dry wt), PO_2 = oxygen tension in mm Hg, and K_1 and K_2 are constants calculated from the linear form of equation (1) or, in more general terms: $Y = X \div (K_1 + K_2X)$.

It is evident that if K_2 is large compared with K_1, then Y will not vary much with X. The limiting case is if K_1 is so small that you can ignore it, in which case X/K_2X is a constant equal to Y.

Similarly, increasing K_1 in relation to K_2 causes greater variation of Y as X varies. It should be noted that as long as K_1 is greater than 0, the equation can never describe complete oxygen independence.

Van Winkle and Mangum (1975) overcame this problem by including an intercept parameter in the equation so that the predicted curve need not pass through the point (X, Y) = (0, 0). Rearranging (1) gives:

$$PO_2/QO_2 = K_1 + K_2 \cdot PO_2 \quad (2)$$

where: K_1 = intercept, and K_2 = slope of the regression equation relating PO_2/QO_2 to PO_2. This provides an empirical means of finding the values of K_1 and K_2.

Bayne (1971) proposed that the ratio K_1/K_2 provides an index of dependence

of oxygen consumption on oxygen tension, since as K_1 increases in relation to K_2 the rate of oxygen consumption, VO_2, becomes more directly proportional to oxygen tension, PO_2 (oxygen dependent species). Conversely, as K_2 increases in relation to K_1, oxygen consumption approaches a constant value (oxygen independent species) for different values of PO_2.

Mangum and Van Winkle (1973) tested a number of equations which might be used to describe the relationship between oxygen consumption and oxygen concentration. The found that for goodness of fit, ease of computation, and useful parameters for interspecific comparisons, a second order polynomial gave the best results:

$$Y = B_0 + B_1X + B_2X^2 \quad (3)$$

where Y = weight specific oxygen consumption ml/(h · g dry wt), X = PO_2, B_0 = minimum rate of oxygen uptake found at very low PO_2, B_1 = linear effect of X on Y, and B_2 = deviation from linearity of the effect of X on Y.

The equation uses standardized data, *i.e.* the initial value is expressed as 1.0 and subsequent values as fractions of 1.0. Thus, for a strict oxyregulator (oxygen independent species) B_0 would equal 1, and B_1 and B_2 would equal zero. A strict oxyconformer (oxygen dependent species) would have both B_0 and B_2 equal to 0 and $B_1 > 0$. Mangum and Van Winkle (1973) also suggested the second order coefficient, B_2, may be used as an index of a species' ability to regulate its rate of oxygen uptake in declining oxygen tensions. The more negative the value of B_2 the more oxygen independent the animal. Both of the above methods make use of the "weight specific" oxygen consumption, QO_2, in their application. Bayne and Livingstone (1977), however, established a treatment for results relating VO_2 (oxygen consumption in ml/h) to PO_2. They fitted both standardized and nonstandardized data relating VO_2 and PO_2 to the three equations shown in Table III. In contrast with the method of Mangum and Van Winkle (1973), these equations were not meant to produce parameters of biological relevance but rather to give a means of relating VO_2 to PO_2 in different experiments.

For comparative purposes and so that the data here might be compared with those already available in the literature, all three approaches have been employed in analyzing the data.

Effect of declining oxygen tension on oxygen consumption at full salinity

A total of 20 experiments relating oxygen consumption to oxygen tension were carried out. The results of six typical experiments are shown in Figure 1. In each

TABLE III

Statistical models used to fit data relating VO_2 to PO_2, and the goodness of fit of these models. K_1, K_2, and K_3 are fitted parameters (after Bayne and Livingstone, 1977).

	Model	Sums of squares of deviations: Non-standardized data	Sums of squares of deviations: Standardized data
Hyperbolic	$VO_2 = \frac{PO_2}{(K_1 + K_2 \cdot PO_2)}$	0.0113	0.2933
Polynomial	$VO_2 = K_1 + K_2 \cdot PO_2 + K_3 \cdot (PO_2)^2$	0.0150	0.2384
Semilogarithmic	$VO_2 = K_1 + K_2 (\log_{10} PO_2)$	0.0171	0.2712

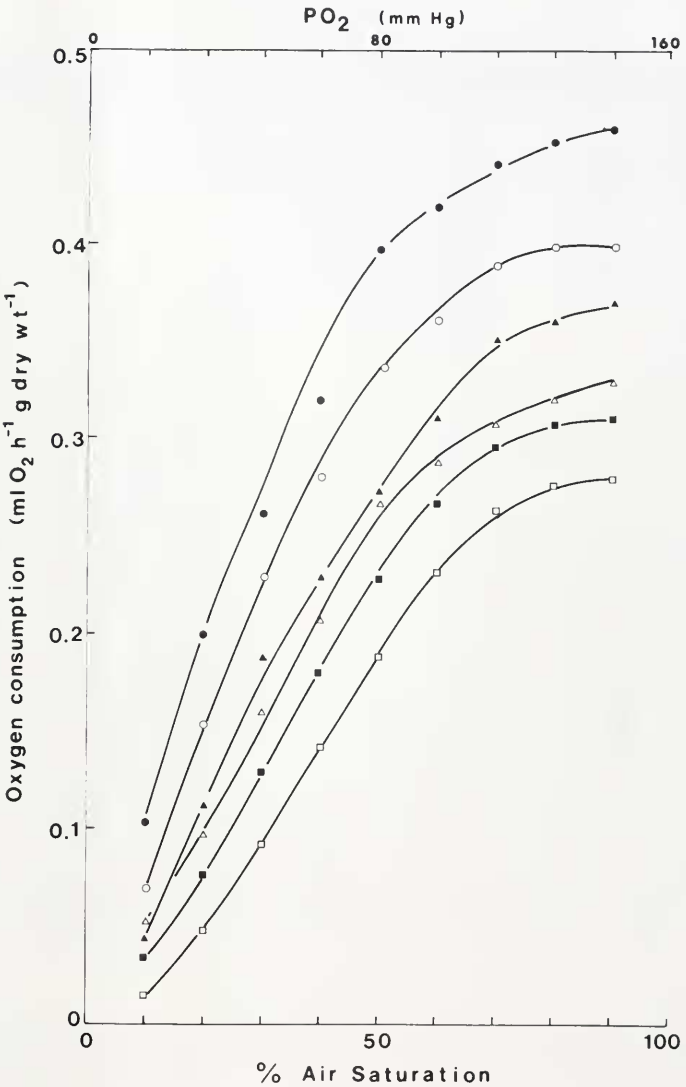

FIGURE 1. Responses of six specimens of *A. crenata* to declining oxygen tension.

experiment the rate of oxygen consumption appeared to be more or less independent of the ambient PO_2 over a narrow range of oxygen tensions.

In order to estimate the degree of this independence, PO_2/QO_2 was plotted against PO_2 (with PO_2 the oxygen tension in mm Hg and QO_2 the weight specific oxygen consumption) (Tang, 1933; Bayne, 1971a, b). As discussed previously, Bayne (1971a, b) proposed the use of the ratio K_1/K_2 as an index of oxygen dependence with smaller values of this ratio, indicating a greater degree of oxygen independence. The values for K_1 and K_2 are given in Table IV.

Figure 2 shows the relationship between the ratio K_1/K_2 and QO_2. The equation of the resultant regression line is: $K_1/K_2 = 527.7QO_2^{1.610}$.

TABLE IV

Values of K_1 and K_2 in the expression $QO_2 = PO_2/K_1 + K_2\ PO_2$ for seven specimens of A. crenata *of various sizes, subjected to declining oxygen tensions. QO_2, ml O_2/(h·g dry wt); PO_2, mm Hg.*

Animal Number	K_1	K_2	K_1/K_2
1	196	2.14	91.59
2	206	1.60	128.75
3	237	1.45	163.44
4	177	1.72	102.90
5	191	1.27	150.39
6	124	1.54	80.52
7	113	1.30	86.92

Small individuals with high metabolic rates are, therefore, less independent of the ambient oxygen tension than are larger individuals with low metabolic rates.

Effect of time spent in the laboratory on the ability to regulate QO_2

Bayne (1971) showed that the length of time the mussel, *Mytilus edulis*, was maintained in the laboratory could affect the animals' capacity to regulate oxygen uptake in declining oxygen tensions. This possibility was therefore investigated in *A. crenata.*

Animals of similar size were kept in the laboratory for periods of 1, 2, 3, 5, 7, 9, and 12 weeks, after which their response to declining oxygen tension was measured. Table V shows the change in the ratio K_1/K_2 for 70 experiments. It can be seen from these values that, like *Mytilus, A. crenata* loses at least part of its ability to regulate its rate of oxygen consumption in declining oxygen tensions if kept in the laboratory for extended periods.

Combined effects of salinity, declining oxygen tension, and anoxia on oxygen consumption

The effect of salinity on oxygen dependence is shown in Table VI where the oxygen dependence index, K_1/K_2, is given for snails from six different salinities.

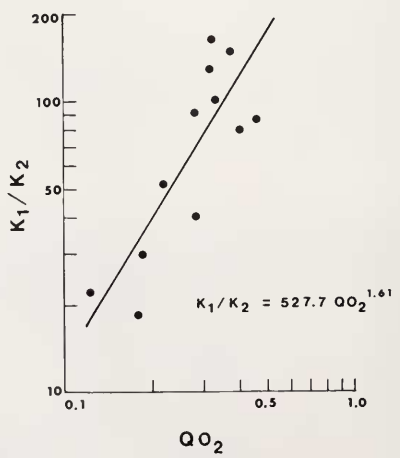

FIGURE 2. The "oxygen dependence index" K_1/K_2, plotted against weight-specific oxygen consumption.

TABLE V

Oxygen-dependence index for specimens of A. crenata *after different periods in the lab. N = 10 for each experiment.*

Time (weeks)	K_1/K_2
1	93.22
2	90.10
3	98.74
5	312.47
7	415.26
9	510.17
12	905.56

Table VII shows B_2 values (see equation 3) for the same salinities. As the salinity decreases, K_1/K_2 also decreases, indicating an increase of oxygen independence with decreasing salinities. B_2 values become increasingly more negative, also indicating increased oxygen independence.

Figure 3 shows the rates of oxygen consumption during declining oxygen tensions at different salinities after 2 h anoxia. As salinity decreases the zone of critical pressure, P_c, shifts left, *i.e.* the animals become more oxygen independent. In animals placed in 125, 100, and 75% sea water (Line A, Fig. 3) the P_c is between 60–70% air saturation. In animals exposed to O and 25% sea water, the P_c is between 40–50% air saturation. This shift is reflected in the increasingly more negative values of B_2 (Table VII). This gives the animals an increased range of oxygen tensions over which they can maintain an elevated rate of oxygen uptake.

Figure 3 also shows the rates of oxygen consumption during declining oxygen tensions at various salinities in animals previously exposed to 12 h anoxia. Again, this shift if reflected in the B_2 values given in Table VII. In this case the P_c has shifted to the left to a value of 30–40% air saturation, increasing even further the range of oxygen tensions over which they can maintain an elevated rate of oxygen uptake.

DISCUSSION

Little information is available concerning oxygen consumption of marine pulmonates. Berg and Ockleman (1959), studying several species of freshwater pul-

TABLE VI

Oxygen dependence index (K_1/K_2) for specimens of A. crenata *at five different salinities. N = 10; dry weight ≃ 0.3 g.*

Salinity (% SW)	Mean QO_2 in air-saturated SW (ml/(h·g dry wt))	Mean K_1/K_2
125	0.48	90.38
100	0.50	96.24
75	0.43	91.46
50	0.58	66.27
25	0.49	31.14
0	0.52	34.61

TABLE VII

B_2 values ($\times 10^3$) from the expression $Y = B_0 + B_1 X + B_2 X^2$ (see equation 3) for specimens of A. crenata *exposed to declining oxygen tensions and to anoxia at various salinities. N = 10 for each experiment.*

Salinity % sea water	B_2 ($\times 10^3$) Normal	After 2 h anoxia	After 12 h anoxia
125	−0.0497	−0.0511	−0.0739
100	−0.0513	−0.0532	−0.0754
75	−0.0502	−0.0567	−0.0740
50	−0.0590	−0.0701	−0.0723
25	−0.0662	−0.0723	−0.0752
0	−0.0681	−0.0721	−0.0749

monates, found the oxygen consumption in relation to weight to vary between less than proportional to surface area and proportional to weight. Brand *et al.* (1948) studied respiration of nine species of freshwater pulmonates with regard to body size but not to the weight exponent b. Their data show an apparent constancy of weight/O_2 relationship within limited size ranges, but also show that the overall picture is distinctly against the validity of such a procedure. For comparative purposes, extrapolating from their graph for oxygen consumption vs. body weight for all pulmonates studied gives an approximate value for b of 0.85. Newell and Pye (1971) report a range of b values for *Littorina littorea* at different temperatures and seasons of 0.245–0.033 for "resting animals." Shumway and Crisp (in preparation) studied oxygen consumption in 30 species of marine gastropods and found values of b ranging from 0.517 to 0.863. The b value of 0.45 reported here for *Amphibola* is relatively low but not unusual and is not affected by salinity changes.

Brand *et al.* (1948) found that the respiratory rate of freshwater pulmonates was higher than that of operculates when snails of equal weight were compared.

In the equation $Y = aW^b$, a is the magnitude of Y (ml/h) when W equals 1 (g dry wt) and can be used to compare oxygen uptake for different animals of the same weight. Shumway and Crisp (in preparation) found "a" values for snails generally similar in size to *Amphibola* to range from 0.128 to 0.166 (*Crepidula fornicata, Calliostoma zizyphinum, Littorina littorea, Patella vulgata*). The value of 0.158 reported here for the marine pulmonate *A. crenata* is within this range and it does not appear that marine pulmonate respiration is any higher than that of marine prosobranchs.

Few authors have studied oxygen consumption of snails in both air and water. Sandison (1966) found that four species of intertidal gastropods all had higher rates of oxygen consumption in air than in water and that in species living at successively higher shore levels the rates of oxygen consumption in air increased. Conversely, McMahon and Russell-Hunter (1977) studied aerial and aquatic oxygen consumption in several species of intertidal prosobranchs and found the aerial uptake rates lower than the aquatic ones in all four intertidal snails studied. Micallef and Bannister (1967) found that aerial and aquatic oxygen consumption in *Monodonta turbinata* were the same at 15°C. They also note that the snail is observed as frequently out of water as immersed. Bannister (1974) measured aerial and aquatic oxygen consumption in *Patella caerulea* (lower eulittoral zone) and *P. lusitanica* (upper eulittoral zone). He found that the rate of oxygen consumption of *P. lusitanica* was about three times higher in air than in water and that the rate of oxygen consumption of *P. caerulea* in water was about twice that in air. Thus,

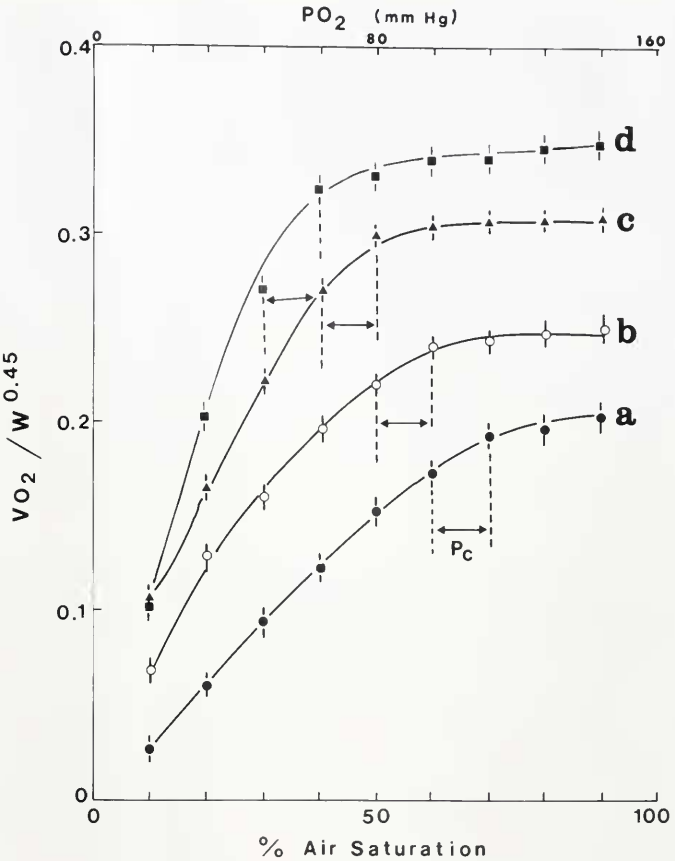

FIGURE 3. The rates of oxygen consumption ($VO_2/W^{0.45}$, ml O_2^{h-1}) by *Amphibola crenata* in declining oxygen tensions (PO_2: mm Hg) at different salinities after two and 12 h exposure to anoxia. Solid circles: 125, 100, and 75% sea water and 2 h anoxia; open circles: 50% sea water and 2 h anoxia; triangles: 25 and 0% sea water and 2 h anoxia; solid squares: all salinities and 12 h anoxia. P_c = zone of critical pressure. N = 10 for each salinity tested.

all three studies indicate that respiratory rates in air and water vary according to whether the animals are exposed or submerged.

Accordingly, it might be expected that *Amphibola* would show a higher rate of oxygen consumption in air than in water. This is not the case (Table I). The one major difference between the snails previously studied and *A. crenata* is that *Amphibola* is a pulmonate, and the others are prosobranchs. *Amphibola* employs the mantle cavity as a lung and has been described as being at a "lung-fish" stage of gastropod evolution (Morton and Miller, 1973). As this snail occupies a transitional habitat between marine and terrestrial conditions it is not surprising that it can use aerial and aquatic oxygen equally well. Possibly evolutionary position, not position on the shore, influences the snail's respiratory capabilities.

Kinne (1971) has divided the effects of salinity on the rate of oxygen consumption in marine and brackish-water invertebrates into four categories: (1) increase in subnormal salinities and/or decrease in supranormal salinities, (2) increase in sub- and supranormal salinities (3) decrease in sub- and supranormal salinities, (4) both remain essentially unaffected.

Several species, predominantly crustaceans, maintain constant rates of oxygen consumption when exposed to different salinities (Krogh, 1939 [*Eriocheir sinensis*]; Gilchrist, 1956 [*Artemia salina*]; Frankenberg and Burbanck, 1963 [*Cyathura polita*]; and McLusky, 1968 [*Corophium volutator*]). The results reported here place *Amphibola crenata* in group (4) also. One other snail, *Theodoxus fluviatilis*, has the same oxygen consumption whether it lives in brackish or fresh water (Lumbye, 1958). In his experiments, Lumbye collected animals from either brackish (11.1‰ salinity) or fresh water and measured the animals' oxygen consumption in the same concentration of sea water from which they were collected. Thus, it is not clear whether snails adapted to life in fresh water would show the same rate of oxygen consumption if exposed to brackish water or vice versa. Possibly, although the species is found in both brackish and fresh water, the two populations are preadapted to life at a particular salinity and not to changes in salinity. Lumbye attributes his findings that both populations have the same rate of oxygen consumption to the fact that *T. fluviatilis* immigrated into fresh water in the early interglacial period, thus giving the animal a longer time to adapt to fresh water than other species that have migrated from the sea to fresh water (*e.g. Potamopyrgus jenkinsi,* first recorded from fresh water in Denmark in 1945) (Lumbye, 1958).

Amphibola crenata, unlike *T. fluviatilis*, is not known from areas of exclusively fresh water, although it is found where it is covered with fresh water for considerable periods. During these periods, the animals carry on their normal activities, including mating and deposition of egg masses (personal observation).

The causes of salinity effects on oxygen consumption rates are not clear. Kinne (1971) states that immediate temporary elevation of oxygen consumption following salinity change may result from an increased overall alertness by the animal to counteract physiological stress. If this is the case, it is not surprising that an animal such as *Amphibola*, which occurs naturally in areas which experience large salinity fluctuations, shows no change in its rate of oxygen consumption when exposed to changing salinities.

Many marine invertebrates tolerate anaerobic conditions for varying periods (Theede, 1973). To survive without oxygen, they must use non-oxidative sources of energy, with the accumulation of end products such as lactic acid, alanine, and succinate (see Mehlman and von Brand, 1951; Livingstone and Bayne, 1977; de Zwaan, 1977). When the animals are returned to aerobic conditions, the by-products of anaerobic metabolism must either be excreted or oxidized. Excretion of all end products will not cause an appreciable increase in oxygen consumption; however, oxidation will increase oxygen demand by tissues over normal metabolic requirements, repaying an oxygen debt. Such payment is a short term phenomena and, as will be seen later, the increased post-anoxic respiratory rates recorded here probably are not related to oxygen debt but rather to a compensatory shift in the respiratory response to low oxygen.

The amount of "oxygen debt" incurred has been related to the species' resistance to anoxia—animals with low resistance showing the greatest oxygen debts. Several species of anemones increase their oxygen consumption after exposure to anoxic conditions (Brafield and Chapman, 1965; Sassaman and Mangum, 1972). In addition, Sassaman and Mangum (1972) found that the burrowing anemone *Haloclava producta* has a survival rate under anoxic conditions at least twice that of the epifaunal anemone *Metridium senile* (even though both species survive anoxia for considerable periods). Mehlman and von Brand (1951) studied 11 species of freshwater snails and found that those with considerable resistance to low oxygen

concentrations appeared to accumulate no oxygen debt after anoxia, whereas those with a low tolerance to anoxia accumlated lactic acid and repaid a large oxygen debt. McMahon and Russell-Hunter (1978) studied six species of marine snails. In the species most likely to encounter low oxygen concentrations in their natural environments, no oxygen debt was repaid after 5 h anoxia. McMahon (1973) has shown that the freshwater limpet *Laevapex fuscus*, commonly found in reducing substrates, incurred no oxygen debt after 48 h anoxia. Bayne and Livingstone (1977) found a positive oxygen debt in only one experiment, using *Mytilus edulis* that had been exposed to hypoxic conditions for 5 h.

Amphibola kept at 100% sea water showed no post-anoxic increase in respiratory rate after 2 h anoxia but showed a gradual increase in the post-anoxic/pre-anoxic respiratory rate between 4 and 24 h anoxia (increase from 1.48–1.76). The post-anoxic respiratory rate was also influenced by salinity. As the salinity of the medium decreased, the post-anoxic/pre-anoxic respiratory rate increased during exposure to short term anoxia (2 h) but no clear pattern was seen for responses evoked after longer periods (4–24 h).

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While *Amphibola* tolerates low oxygen tensions and anoxic conditions, it probably does not regularly encounter long periods of total anoxia in its natural environment. Briggs (1972) estimated that the snails were only covered for approximately 1–2 hours in any one tidal cycle. We have already seen (Table II) that post-anoxic respiratory rates do not increase appreciably over this period unless the salinity is extremely low—unlikely during a flowing tide. During low tide the snails respire in air. Thus, the snails seem to be well adapted for short periods of anoxia.

The maximum ratio of post-anoxic:pre-anoxic respiration recorded never exceeded approximately 1.8, *i.e.* the rate of oxygen consumption after anoxic exposure was 1.8 times higher than the initial rate. Oxygen consumption probably becomes diffusion-limited beyond this point, and respiration cannot proceed any faster.

The relationship between oxygen consumption and tension has been described by calling animals either oxyregulators (oxygen consumption independent of ambient oxygen concentration) or oxyconformers (oxygen consumption dependent on ambient oxygen concentration). As pointed out by Mangum and Van Winkle (1973) few species exactly fit either category. Molluscs range from conformity to regulation (Von Brand and Mehlman, 1953; Bayne, 1967, 1971a, b; Kushins and Mangum, 1971, McMahon, 1973; Mangum and Van Winkle, 1973; McMahon and Russell-Hunter, 1974; Akerlund, 1974; Taylor and Brand, 1975a, b; Bayne *et al.*, 1976; Bayne and Livingstone, 1977; McMahon and Russell-Hunter, 1978). Bayne (1971a), and Mangum and Van Winkle (1973) put forth means of dealing with these intermediate curves (see results section). The polynomial provides the best fit for standardized data in agreement with the findings of Mangum and Van Winkle (1973) (Table III). The B_2 ($\times 10^3$) value for *A. crenata* was −0.0513, intermediate between the values reported by Mangum and Van Winkle (1973) for two species of gastropods (*Lunatia heros* 0.0489 and *Urosalpinx cineria* −0.1180). McMahon and Russell-Hunter (1978) report values of B_2 for six species of snails ranging from −0.0403 to −0.0868. Table VIII gives the K_1/K_2 values for *Amphibola*

TABLE VIII

The regression equations for the oxygen-dependence index, K_1K_2, against the weight specific oxygen consumption QO_2.

Species	Regression equation	Number of determinations	Source
Geloina ceylonica	$K_1/K_2 = 3.88QO_2^{0.457}$	10	Bayne (1973)
Anadara granosa	$K_1/K_2 = 6.46QO_2^{0.615}$	7	Bayne (1973)
Modiolus demissus	$K_1/K_2 = 62.71QO_2^{0.930}$	10	Shumway (1979)
Mytilus edulis	$K_1/K_2 = 75.50QO_2^{0.818}$	10	Bayne (1971)*
Chlamys delicatula	$K_1/K_2 = 115.78QO_2^{0.769}$	6	McKay & Shumway (1980)
Laevicardium crassum	$K_1/K_2 = 550.00QO_2^{1.829}$	10	Bayne (1971)*
Amphibola crenata	$K_1/K_2 = 527.70QO_2^{1.610}$	12	Present study

* See Mackay and Shumway (1980).

along with values recorded for other species of molluscs. As in other species studied (Bayne, 1971a, b; Taylor and Brand, 1975b; Mackay and Shumway, 1980) it was found that the ratio K_1/K_2 for *A. crenata* declines with decreasing QO_2 and, therefore, with increasing body size, *i.e.* the animals show an increase in respiratory independence with increasing size. This is reflected in the snail's distribution: Small animals (with little or no capabilities for respiratory independence) are found predominantly in well-aerated, high-salinity water.

Both the large K_1/K_2 value and B_2 value indicate that *Amphibola* has little capacity for regulating its rate of oxygen consumption in 100% sea water without prior anoxic stress.

As pointed out by Lange *et al.* (1972), many studies deal with the effects of declining oxygen tension on oxygen consumption, but relatively few discuss the effects of other factors such as salinity and previous exposure to anoxia on the animals' response to declining oxygen tension.

Bayne (1973) studied the responses of three species of bivalve molluscs to declining oxygen tension at reduced salinity. In all three species, capacity to regulate oxygen consumption in declining oxygen tension decreased with reduced salinity, *i.e.* the ratio K_1/K_2 increased with decreasing salinity. This is opposite to the response shown by *A. crenata* (Table VI), where the ratio K_1/K_2 decreased with decreasing salinity, indicating an increased capacity for regulating oxygen consumption with decreased salinity. This difference may not be as startling as it appears. Bayne (1973) also points out that while the stenohaline species, *Anadara*, cannot maintain a high oxygen uptake in declining oxygen tensions at reduced salinities, two more euryhaline species, *Geloina* and *Mytilus*, maintained higher rates of oxygen uptake under the same conditions. *Amphibola* has a "preferred" salinity equal to approximately 60% sea water, (Shumway and Freeman, in prep.) and it is therefore perhaps not surprising that this species shows maximum capacity to regulate oxygen consumption at salinities of 0–15‰ (0–50% SW) and loses the capacity in salinities above approximately 15‰ (50% SW). There also is no change in K_1/K_2 for *Amphibola* in the range 75–125% sea water.

Van Winkle and Mangum (1975) pointed out that temperature or salinity might

modify the value of B_2. Table VII indicates that in *A. crenata*, the lower the salinity, the lower the value of B_2. Again, this indicates increased regulation in decreased salinities.

McMahon and Russell-Hunter (1978) studied the respiratory responses to low oxygen stress in six species of snails exposed to varying periods of anoxia. Pre-stress respiratory patterns shifted to a post-stress pattern of greater oxygen independence, particularly at low oxygen tensions. These authors feel that post-stress increases in oxygen uptake rates and greater regulation of oxygen uptake are a short-term compensatory response that allows the snails to maintain relatively high aerobic metabolic rates, instead ot switching to less efficient anaerobic pathways, during occasional low oxygen concentrations. *A. crenata* (see Fig. 3) responded similarly to 12 h anoxia. The zone of critical oxygen pressure shifted from about 60–80% to about 30–40% air saturation. It is presumed that this response, like the one previously reported by McMahon and Russell-Hunter, is short-term, allowing the snails to maintain high rates of oxygen consumption in declining oxygen tensions. (for instance, when repaying an oxygen debt).

An animal such as *Amphibola* may encounter anoxia and salinity stress simultaneously in its natural environment. Salinity did not affect oxygen consumption by *A. crenata* at concentrations near oxygen saturation, but post-anoxic oxygen consumption depended on salinity as well as length of anoxic exposure. After a 2 h anoxia (Fig. 2, 3), salinity has a pronounced effect on the snails' capacity to regulate oxygen consumption in declining oxygen tensions. Over this period, as the salinity decreases, the zone of critical pressure shifts to the left, so that for maximum salinity stress (0–25% sea water) the zone of critical pressure has shifted to about 40–50% air saturation. Since these snails are unlikely to be exposed to anoxia for more than about 2 h at any one time, this shift, too, probably is a short-term response enabling the animals to avoid reverting to anaerobic metabolism.

After 12 h anoxia (*i.e.* when the post-anoxic/pre-anoxic respiration rate is at its maximum) salinity no longer prominently affects the zone of critical pressure and the P_c has shifted even farther to the left, into about 30–40% air saturation, for all salinities tested. This is also reflected in the values for B_2 for varying periods of anoxic stress (Table VII).

In *Amphibola*, the long-term post-anoxic elevations in oxygen consumption apparently are not related to an "oxygen debt" payment but rather to compensatory shifts in the respiratory response to low oxygen, involving increased oxygen consumption at all oxygen tensions and a marked shift toward oxygen independency (see Fig. 3). Such respiratory compensation has been described for *Mytilus edulis* (Bayne, 1973) and for several species of prosobranchs (McMahon and Russell-Hunter, 1978). It should also be noted that longer periods of anoxia increase the rate of oxygen consumption at full oxygen saturation and that long-term anoxia causes greater shifts toward regulation of oxygen consumption. Thus, in *Amphibola crenata*, salinity and anoxia both affect the response to declining oxygen tensions in the short-term, whereas only anoxia affects the respone in the long-term.

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